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EXUDATE FLAVONOIDS FROM TWO AUSTRALIAN ASTERACEAE, *BRACTEANTHA VISCOSA* AND *CASSINIA QUINQUEFARIA*

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Key Word Index—*Bracteantha viscosa*; *Cassinia quinquefaria*; Asteraceae; leaf and stem exudates; flavonoid aglycones; 6-hydroxygalangin; rare flavanones.

Abstract—Nineteen flavonoids were identified in the leaf and stem exudates of *Bracteantha viscosa*: 12 flavones and seven flavanones. Two flavones and four flavanones are rare 6-methoxyflavonoids. The resinous exudate accumulating on aerial parts of *Cassinia quinquefaria* contains six flavonoid aglycones. One of them is a novel natural flavonol: 6-hydroxygalangin.

INTRODUCTION

Most of the higher plants that we have studied so far for the presence of externally accumulated flavonoid aglycones [1] were from Northern Mexico and the southwest of the U.S.A., or from the Mediterranean. We have now analysed the exudate flavonoids of two plants from Australia, namely *Bracteantha viscosa* (DC.) A. Anderb. and L. Haegi and *Cassinia quinquefaria* R.Br. The genus *Bracteantha* consists of five species all endemic to Australia. Several species and varieties have been widely cultivated, and are known as 'everlastings' because of the dry papery bracts surrounding the flower heads. *Bracteantha viscosa* (previously *Helichrysum viscosum* [2]) grows as an erect perennial herb, usually 40–50 cm high, with viscid and scabrid leaves. Its natural distribution is in southeastern Australia, occurring on the slopes and higher tablelands of southern Queensland, New South Wales and Victoria. *Cassinia* R.Br. in its present sense [2] is composed of *ca* 21 species occurring in Australia and New Zealand. *Cassinia quinquefaria* is a diffuse shrub growing to about 3 m high with viscid resinous leaves. It occurs in southeastern Australia, in southern Queensland and the eastern coast and tablelands regions of New South Wales. Both *Bracteantha viscosa* and *Cassinia quinquefaria* are summer flowering plants that grow mostly in the understorey of open dry sclerophyll forests and eucalypt woodlands.

RESULTS AND DISCUSSION

In the exudate of *Bracteantha viscosa* a series of flavonoid aglycones was identified unambiguously by direct comparisons with markers: apigenin, apigenin 7-methyl ether, scutellarein 6-methyl ether, scutellarein 6,7-dimethyl ether, scutellarein 6,4′-dimethyl ether, luteolin, luteolin 7,3′-dimethyl ether, 6-hydroxyluteolin 6-methyl ether, 6-hydroxyluteolin 6,3′-dimethyl ether, 5,4′-dihydroxy-6,7,8-trimethoxyflavanone (xanthomicrol), naringenin, 5,4′-dihydroxy-6,7-dimethoxyflavanone (methoxysakuranetin), eriodictyol, eriodictyol 3′-methyl ether and 8-methoxyeriodictyol. Found for the first time in leaf glands of *Plectranthus ecklonii* [3] and in the frond exudate of the fern *Cheilanthes argentea* [4], 6-methoxysakuranetin is a rare natural product; it has since been found in aerial parts of *Sideritis sventenii* [5]. Only once before has 8-methoxyeriodictyol been reported, namely in the exudate of *Encelia frutescens* var. *resinosa* [6]. It is also present in the exudate of *Ambrosia bidentata* (Wollenweber *et al.*, unpublished results).

Compound **1** showed the molecular mass ($[M]^+$, 374) of a flavone/flavonol with two hydroxy and four methoxy groups. That ($[M]^+ - 15$) was much more intense than $[M]^+$ suggested 8-methoxy substitution. The ^{1}H NMR spectrum revealed a low-field singlet characteristic of the strongly H-bonded 5-OH and three singlets in the aromatic region, one of which integrated for two protons. This suggested a tri-substituted A-ring and a 3′,4′,5′-trisubstituted B-ring. The three methoxy signals, one of which integrated for six protons, indicated that two methoxyls were attached symmetrically to the B-ring (3′ and 5′). The ^{13}C NMR spectrum also showed three methoxyl signals: one of double intensity, three CH signals in the aromatic region (one of double intensity; C-2′ and C-6′) and 11 signals from quaternary carbons (one of double intensity; C-3′ and C-5′). The A- and B-ring signals are very similar to those of 5,7-dihydroxy-3,8,3′,4′,5′-pentamethoxyflavone [7] and the C-ring

signals are similar to those of 5,7,3′-trihydroxy-6,8,4′-trimethoxyflavone (acerosin) [8]. Compound **1** is, therefore, 5,7-dihydroxy-8,3′,4,5′-tetramethoxyflavone, a substance reported previously only as a constituent of 'dikamali gum', the resinous exudate of *Gardenia lucida* [9].

Compound **2** is a flavanone with three hydroxy groups and one methoxy group ([M]$^+$, 302). Its ^{1}H NMR spectrum showed the typical ABX system of three protons corresponding to the C-ring in a flavanone (multiplets at 2.72, 3.17 and 5.42 ppm). Two doublets (two protons each) and a one-proton singlet were observed in the aromatic region. The 9 Hz coupling between the aromatic doublets indicated *para*-substitution in the B-ring. The peak at *m/z* 120 in the MS, arising from RDA fragmentation of the C-ring, confirmed the presence of a B-ring hydroxyl. The remaining two hydroxyls and one methoxyl are thus located on the A-ring, and the observed singlet at 5.99 ppm indicates H-8. By comparison with reported data, **2** was identified as 5,7,4′-trihydroxy-6-methoxyflavanone (6-methoxynaringenin), a very rare flavanone, found previously only in the root of *Scutellaria baicalensis* [10], in flowers of *Tanacetum sibiricum* [11] and in aerial parts of *Hymenoxys turneri* [12].

Compound **3** is a flavanone with four hydroxy groups and one methoxy group ([M]$^+$, 318). Its ^{1}H NMR spectrum is closely related to that of **2**, except that the B-ring doublets have been replaced by two multiplets at 7.02 ppm (one proton) and 6.85 ppm (two protons) indicative of 3′,4′-substitution. The additional hydroxy group must thus be located at 3′ and was confirmed by the corresponding RDA fragmentation peak (*m/z* 136). Compound **3** was thus identified as 6-methoxyeriodictyol, which was first reported from *Filifolium sibiricum* [13] and named filifolin. It has since been found in the aerial parts of *Gutierrezia sphaerocephala* [14], *Eupatorium subhastatum*[15] and *Hymenoxys turneri* [12].

In the exudate of *Cassinia quinquefaria*, pinocembrin, pinobanksin, pinobanksin 3-acetate, galangin and 6-methoxygalangin were identified by direct TLC comparisons with markers. Identifications were further confirmed by melting points and/or mass spectra. The rather polar **4** appeared on polyamide TLC as a dark spot that turned dark brown after spraying with the Naturstoff reagent. Its MS exhibited [M]$^+$ at *m/z* 286, indicating a flavone/flavonol with four hydroxy groups. UV-shifts with classical reagents indicated a 3,5-dihydroxyflavonol ($AlCl_3$, $AlCl_3$+HCl) with a 7-hydroxy group in the presence of 6-oxygenation (NaOAc). These data suggested the structure of 6-hydroxygalangin for **4**, which was confirmed by the ^{1}H and ^{13}C NMR data. The ^{1}H NMR spectra of 6-methoxygalangin and of **1** have superimposable signals in the aromatic region. The spectrum of 6-methoxygalangin had an appropriate Ar-OMe signal which was absent from the spectrum of **4**. The signal for a H-bonded OH at C-5 was present in the spectrum of **4**. Finally demethylation of 6-methoxygalangin with pyridinium bromide afforded a product identical to **4**. This flavonol was thus confirmed as 6-hydroxygalangin, reported here for the first time as a natural product. Its occurrence in the exudate of *Cassinia quinquefaria* has been mentioned earlier in a review [16].

EXPERIMENTAL

Aerial parts of *Bracteantha viscosa* were collected by J. G. West at Black Mountain near Canberra, Australia (35° 16′S, 149° 07′E). Vouchers are deposited in the Australian National Herbarium in Canberra, A.C.T. (J.G.W. 5130). Aerial parts of *Cassinia quinquefaria* were collected on 20.02.87 by J. G. West and on 5.11.87 by J. Palmer in the same locality (J.G.W. 5128 and J. Palmer 125). Air-dried plant material was briefly rinsed with acetone to dissolve the exudate. Concentrated solns were processed by CC on Sephadex LH-20 (eluted with MeOH), followed by CC on silica and/or polyamide SC-6 (eluted with toluene and increasing amounts of MeCOEt and MeOH). Frs were monitored by TLC on silica with solvents: A (toluene–MeCOEt, 9:1) and B (toluene–MeCOEt–HOAc, 18:1) and on polyamide DC-11 with solvents C (petrol bp 100–140° – toluene–MeCOEt–MeOH, 12:6:1:1); D (toluene–petrol bp 100–140°–MeCOEt–MeOH, 12:6:2:1) and E (toluene–MeCOEt–MeOH, 12:5:3). Chromatograms were viewed in UV_{366} before and after spraying with 'Naturstoffreagenz A' (NA; for phenols) and with $MnCl_2$ reagent (for terpenoids; used on silica only). Phenolic frs were combined according to the major constituents observed, which then crystallized from the concd solns. Some were isolated by prep. TLC on precoated silica plates. Identification of known flavonoids was by direct comparison with markers, supported by mp, UV and MS data. Most markers were available in E.W.'s lab.; 6-methoxygalangin [17] and 6-methoxynaringenin [18] were synthetic samples. Rare and new flavonoids were identified by spectroscopic methods including NMR. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively. MS were obtained at 70 eV. Mps: uncorr.

In the exudate of *Bracteantha viscosa* a series of flavonoid aglycones was identified unambiguously by direct comparison with markers. Several less common flavones and flavanones were further characterized by their mass and NMR spectra. We thus identified 5,7,4′-trihydroxy-6,8-dimethoxyflavone, 5,7-dihydroxy-8,3′,4′,5′-tetramethoxyflavone (**1**), 6-methoxynaringenin (**2**), 6-methoxyeriodictyol (**3**) and 8-methoxyeriodictyol.

Compound **1** is a yellow solid product. UV λ_{max}^{MeOH} nm: 330, 277, +$AlCl_3$ 347, 285, unchanged with HCl; NaOH 382, 285; NaOAc 330, 282, unchanged with H_3BO_3. MS *m/z* (rel. int.): 374 ([M]$^+$, 53%), 359 (100), 344 (23), 329 (16), 139 (14), 69 (11). ^{1}H NMR δ (ppm): 12.52 (*s*, 5-OH); 7.37 (*s*, H-2′/H-6′); 7.12 (*s*, H-3); 6.31 (*s*, H-6), 3.92 (*s*, 3-OMe/5-OMe); 3.88* (*s*, 4-OMe); 3.77* (*s*, 8-OMe). ^{13}C NMR δ (ppm): 162.5 (C-2); 104.7 (C-3); 181.9 (C-4); 156.1 (C-5); 98.9 (C-6); 157.2 (C-7); 127.5 (C-8); 149.4 (C-9); 103.5 (C-10); 125.9 (C-1′); 103.7 (C-2′/C-6′); 153.1 (C-3′/C-5′); 104.6 (C-4′); 61.0 (OMe-8); 56.0 (3′-OMe/5′OMe); 60.1 (4′-OMe). (*Interchangeable signals.)

Compound **2**, MS *m/z* (rel. int.): 302 ([M]$^+$, 70%), 182 (74), 167 (60), 154 (25), 153 (19), 139 (17), 137 (12), 120

(20), 91 (21), 69 (100). ^{1}H NMR δ (ppm): 12.31 (*s*, 5-OH); 7.38 (*d*, J = 8.7 Hz; H-2′/H-6′); 6.88 (*d*, J = 8.7 Hz; H-3′/H-5′); 5.99 (*s*, H-8); 5.42 (*dd*, J = 13, 3 Hz; H-2b); 3.77 (*s*, OMe); 3.17 (*dd*, J = 17, 13 Hz; H-3α); 2.72 (*dd*, J = 17.3, 3 Hz; H-3β). NMR data are identical with those of a synthetic sample [18].

Compound **3**, MS *m/z* (rel. int.): 318 ([M]$^+$, 100%), 183 (78), 182 (89), 167 (76), 154 (28), 136 (26), 69 (50). ^{1}H NMR δ (ppm): 12.30 (*s*, 5-OH); 7.02 (*bs*, H-2′); 6.85 (*bs*, H-5′/H-6′); 5.99 (*s*, H-8); 5.36 (*dd*, J = 13, 3 Hz; H-2b); 3.76 (*s*, OMe); 3.17 (*dd*, J = 17, 13 Hz; H-3a); 2.71 (*dd*, J = 17, 3 Hz; H-3b).

8-Methoxyeriodictyol, MS *m/z* (rel. int.): 318 ([M]$^+$, 77), 183 (89), 182 (79), 167 (90), 154 (35), 153 (38), 139 (30), 136 (33), 69 (100).

Work-up of *Cassinia quinquefaria* exudate yielded pinocembrin (mp 195–196°, HOAc), pinobanksin (mp 174–176°, MeOH), pinobanksin 3-acetate (mp 159–161°, EtOH), galangin (mp 221–222°, HOAc), 6-methoxygalangin (mp 237–240°) and **4** as crystalline solids.

Compound **4** precipitated from HOAc as a light yellow powder, mp 250–252°. UV λ_{max}^{MeOH} nm: 336, 275 + $AlCl_3$ 400, 287 (increase); $AlCl_3$ + HCl (405), 373, 281; NaOH 387, 297 (slight decrease); NaOAc 380, 282, 257; NaOAc + H_3BO_3 375, 258. MS *m/z* (rel. int.): 286 ([M]$^+$, 100), 272 (16), 254 (19), 153 (26), 105 (12). ^{1}H NMR (DMSO-d_6) δ (ppm): 12.13 (*s*, OH-5); 8.16 (*dd*, J = 2, 8 Hz, H-2′, H-6′); 7.55 (*m*, H-3′/H-4′/H-5′); 6.58 (*s*, H-8) ^{13}C NMR (DMSO-d_6) δ (ppm): 145.6* (C-2); 136.7 (C-3); 176.3 (C-4); 145.8* (C-5); 128.7 (C-6); 154.0 (C-7); 93.5 (C-8); 149.1 (C-9); 103.6 (C-10); 131.2 (C-1′); 127.6 (C-2′/C-6′), 128.6 (C-3′/C-5′), 129.9 (C-4′). [6-OMe-Galangin for comparison: ^{1}H: 8.16 (*dd*, J = 2, 8 Hz, H-2′/H-6′); 7.55 (*m*, H-3′/H-4′/H-5′); 6.58 (*s*, H-8); 3.77 (*s*, OMe-6); ^{13}C: 145.9 (C-2); 136.8 (C-3); 176.4 (C-4); 151.7† (C-5); 131.0* (C-6); 157.7 (C-7); 93.9 (C-8); 151.8† (C-9); 103.6 (C-10); 131.0* (C-1′); 127.6 (C-2′/C-6′); 128.6 (C-3′/C-5′); 130.0 (C-4); 60.0 (C-OMe). (*†Interchangeable signals.)]

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Note added in proof

6-Hydroxy galangin has recently been reported as a constituent of *Platanus acerifolia* buds: Kaouadji, M., Chiron, S., Garcia, J., Thomasson, F., Tissut, M. and Ravanel, P. (1992) *Phytochemistry* **31**, 2131. In that paper, UV and NMR data are given only for its acetate.